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THE LABORATORY DIAGNOSIS OF VIRAL DISEASE*

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Laboratory methods for the diagnosis of viral diseases may be divided into three broad categories: (1) microscopic methods; (2) serologic methods; and (3) virus isolation procedures.

1. MICROSCOPIC METHODS

This category includes the examination of imprint preparations or smears, as well as examination of fixed and stained tissues. Smear or imprint preparations made from tissues, fluids, exudates, etc., are examined for the presence of inclusion bodies, abnormal cells, and even for the viral or rickettsial agent itself. (Only the rickettsiae and the larger viruses are visible under the compound microscope.)

Fixed tissues are examined for the presence of histologic alterations pathognomonic of specific viral entities. In addition to the staining methods used in morbid anatomy, the virologist uses certain procedures devised to stain the causal agent itself, or the inclusion bodies that it produces, etc.

Histopathologic methods have a very limited application in diagnostic virology because their ability to identify the causal agent on the basis of the histopathologic alterations produced is rather sharply circumscribed. For example, in the encephalitides the tissue changes produced in the brain by a number of viral agents are virtually indistinguishable, not only from each other, but from those induced by a number of other micro-organisms. Similarly, the pathologic changes produced in the lungs by a number of agents known to give rise to the clinical and roentgenographic picture of viral pneumonia are not sufficiently distinctive, on the whole, to permit identification of a specific etiologic virus.

Because of the limitations of microscopic methods, it is highly desirable that material should be *appropriately collected* and stored for subsequent attempts at virus isolation for purposes of substantiating the findings obtained by the microscopic examination. Lest it go unremarked, I should like to stress "appropriately collected." Wherever possible, the tissue specimen should be taken with sterile instruments under aseptic conditions.

Histopathologic methods are of assistance in the diagnosis of a number of viral diseases; for example, rabies, lymphogranuloma venereum, molluscum contagiosum, yellow fever, and some of the exanthemata such as variola, vaccinia, and herpes simplex. With few exceptions, imprint preparations or smears serve as well as tissue sections, and since they are less laborious and time-consuming to prepare, they are preferred over tissue sections. Perhaps the best known application of smears or imprint preparations to diagnosis is in connection with rabies. Infections with this virus are revealed by the presence of pathognomonic structures known as Negri bodies, which occur in the brain and especially in the hippocampus (Ammon's horn). Since a definitive diagnosis by microscopic techniques cannot always be made, it is recommended that the findings, whether positive or negative, be confirmed by virus isolation or by serologic tests or both.

2. SEROLOGIC METHODS

Because serologic methods are relatively simple, rapid, and inexpensive, they are preferred to virus isolation attempts which, even with the recent advances in methodology, still require research procedure such as in the preparation of tissue culture antigens. Most of the tests performed in a diagnostic laboratory are serologic.

I think it necessary to emphasize that the virologist has as great a need as does the clinician for a good clinical end epidemiologic history on which to base his choice of tests and to interpret laboratory findings. Frequently, even the most basic information is not supplied.

Another very important point is the need to recognize that serologic diagnosis depends upon the examination of *two or more* specimens of blood taken at the proper intervals during the course of the patient's illness. Reference to Table 1 will show that during the acute phase of the patient's illness, i.e., within a few days, or even within hours after the onset of symptoms, antibody to certain diseases may be encountered,

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Table 1. Relationship between the stage of illness and the presence of demonstrable virus or antibody in the patient.

Stage of illness	*Virus demonstrable in test material*	*Specific antibody present in serum*
Incubation period	Rarely	
Prodromal period	Rarely	
Onset	Frequently	
Acute phase	Frequently	Frequently*
Recovery phase	Rarely	Generally
Convalescent phase	Very rarely	Usually

* Antibody may be present as a result of prior experience with the virus through infection or vaccination (e.g., influenza) or antibody formation to the infecting virus may be well under way by the time the acute-phase blood is taken (e.g., neutralizing antibody in poliomyelitis and Western equine encephalitis).

although in certain other diseases antibody is absent at this time, i.e., it has not yet had an opportunity to develop. Antibody has appeared or has increased in titer by the time the patient enters the recovery phase of the illness, and by the time convalescence is reached one almost invariably expects to find antibody present in the serum. From these two statements, it follows that if antibody is not detectable during the acute phase of the illness, diagnosis will depend upon the subsequent demonstration of the appearance of antibody between the acute and convalescent stages of the illness. Since, as will be illustrated, the significance of any antibody encountered during the acute phase of the illness cannot be unequivocally assessed, diagnosis in such situations will depend upon detection of a rise in specific antibody titer during the course of the disease. To detect the appearance of antibody or to detect the rise in antibody titer thus requires the examination of two or more specimens of blood taken at appropriately spaced intervals during the course of the illness. The interval between the bleedings should cover a sufficient temporal span to permit antibody formation. This is generally 10-14 days, although it may be as long as 21-28 days, and is sometimes even more, depending upon the disease. To be diagnostically significant, the rise in antibody titer between the acute- and convalescent-phase bleedings should be at least fourfold, i.e., the increase in titer should be of such a magnitude that the antibody level in the convalescent-phase blood is at least four times greater than the level found in the acute-phase blood.

Let us now consider the diagnostic application of the several serologic tests and the principles on which they are based.

Agglutination Tests

Agglutination tests are divisible into two major categories: biologically specific and nonspecific. The biologically specific tests are those in which there is interaction between specific antigen and specific (homologous) antibody. The biologically nonspecific tests are those in which the antigen employed in the test has no etiologic relationship to the disease for which it is diagnostically useful but does react with some substance or substances which appear in the patient's blood during the course of the disease process.

Biologically Specific

The biologically specific agglutination tests find relatively little application in diagnosis since other procedures, such as the complement fixation test, generally serve the purpose better. While macroscopic agglutination tests can be performed with the larger viruses, such as those of vaccinia and psittacosis, and microscopic flocculation tests can even be performed with very small viruses, such as poliomyelitis, complement fixation tests for the diseases produced by these agents are simpler to perform and utilize far less of the expensive antigen. Complement fixation tests are generally employed for the diagnosis of rickettsial diseases, but recently agglutination techniques have been described which appear to be appreciably more sensitive for the detection of antibody than is the fixation method.

Biologically Nonspecific

The Weil-Felix technique is perhaps the best known of the biologically nonspecific agglutination tests. It is used for the diagnosis of rickettsial diseases which fall into the spotted fever and the typhus fever complexes. Although a biologically specific complement fixation test is available for these diseases, the Weil-Felix test is still widely used. It depends upon the fact that the sera of patients suffering from diseases within these two broad groups develop in their blood the so-called "X" agglutinins, i.e., agglutinins for the X strains of *Proteus vulgaris*. The Weil-Felix test is now used in metropolitan laboratories primarily to examine sera which cannot be tested by the complement fixation method because they are anticomplementary or give nonspecific results. The Weil-Felix test not infrequently is positive in infectious conditions other than the rickettsial diseases mentioned, and hence the significance of a positive test must always be assessed in terms of the clinical picture presented by the patient.

Other examples of biologically nonspecific agglutination tests are the cold agglutination test and the streptococcus MG agglutination test, both used in the diagnosis of primary atypical pneumonia. These tests are based on the observation that many patients, during the course of primary atypical pneumonia, develop agglutinins which cause clumping of human group O erythrocytes at icebox temperature and may also develop agglutinins for streptococcus MG.

It should be remembered that inasmuch as the cold agglutination and streptococcus MG agglutination tests are biologically nonspecific, they may be positive in diseases other than primary atypical pneumonia as, for example, gastroenteritis of children (streptococcus MG) and filariasis (cold agglutinins).

Hemagglutination—Inhibition Tests

It has been observed that certain viruses—influenza, mumps, smallpox and several others—possess the capacity to agglutinate certain mammalian and/or fowl erythrocytes and that specific antibody has the capacity to inhibit such hemagglutination. The reaction, therefore, can be utilized on one hand in the identification of viruses and, on the other hand, for the detection and titration of specific antibody.

The hemagglutination - inhibition test is performed by preparing serial

dilutions of a serum under test and adding a standardized amount of virus to each serum dilution. The mixtures are incubated and red blood cells of the appropriate animal species, usually human group O or chick erythrocytes, are added as an indicator system to detect the presence of free virus. In those mixtures where union of specific antibody and virus has occurred, the union will be marked by the absence of agglutination, i.e., hemagglutination is inhibited; where no union has occurred, or virus is in excess, hemagglutination will be present.

The technique appears to be simple of execution and so it is. From the standpoint of the diagnostic laboratory, however, the test has serious drawbacks with respect to the interpretation of the results. Thus, the test is affected in varying degree by nonspecific inhibitors present in serum; unless this is constantly kept in mind, the results obtained may be highly misleading. The hemagglutination-inhibition test perhaps finds its widest application in connection with influenza. All serum specimens to be examined for hemagglutination-inhibiting antibody to influenza virus must generally be treated with trypsin or sodium periodate to destroy nonspecific inhibitory substances prior to testing of the specimens for specific antibody. Again, with reference to influenza, the hemagglutination-inhibition test possesses marked strain-specific reactivities, i.e., hemagglutination-inhibiting antibodies to one strain of influenza virus may show markedly less or even very little inhibitory action against another strain of the virus. In practice, therefore, not only must steps be taken to destroy nonspecific inhibitors in the serum under test but since the test has marked strain-specific reactivities, several different strains of influenza virus have to be employed to be sure that a sufficiently broad spectrum of test hemagglutinins is provided. The complement fixation test, which avoids all these time-consuming manipulations, is generally preferred to the hemagglutination-inhibition test in the diagnostic laboratory. In contrast to the hemagglutination-inhibition test, the complement fixation test has primarily a type-specific reactivity, and is not affected to any practical extent by the antigenic makeup of the virus strains.

However, the strain-specific reactivity of the hemagglutination-inhibition test makes this procedure useful in sorting out and classifying influenza viruses into the several families within a virus group.

Complement Fixation Tests

The complement fixation technique represents the mainstay of the diagnostic laboratory because it is a relatively simple and inexpensive method which lends itself readily to large-scale diagnostic work. This is the principle on which it is based:

The serum of man and animals contains a thermolabile substance known as complement because it complements or completes the action of antibody as, for example, in the destruction of bacteria, the lysis of red blood cells, etc. In the complement fixation test, the serum to be examined is heated to inactivate, or destroy, whatever complement may be present so that it will not affect the results of the test in which measured amounts of complement, usually in the form of guinea pig serum, are used. In performing the test, antigen (virus or viral derivative) and complement are added to serial dilutions of the serum under examination. Complement in such mixture is bound into the complex formed by union of specific antibody with specific antigen. Since evidence of the reaction is not visible, macro- or microscopically, an indicator system has to be used to determine whether antigen-antibody union has occurred. This is effected through the use of mixture of sheep erythrocytes and specific anti-sheep-cell antibody known as hemolysin. The occurrence or nonoccurrence of hemolysis upon the addition of the sheep cell-hemolysin mixture to the reacting system provides evidence as to whether or not antigen-antibody union has occurred in that system, i.e., whether antibody homologous to the antigen was present in the serum of the patient examined.

Complement fixation tests are available for a variety of diseases including influenza A, influenza B, mumps, Newcastle disease, herpes simplex, Colorado tick fever, many of the arthropod-borne encephalitides such as Western equine encephalomyelitis and St. Louis encephalitis, the adenovirus group of infections and, more recently, poliomyelitis. While the technical details of the manner in which the test is performed may vary from laboratory to laboratory, the basic principle is nevertheless the same. However, it should be remembered that because of these variations in technique, the antibody titers obtained in one laboratory may be different from those obtained by another. *The important end result is not the magnitude of the antibody titer, but whether or not a significant rise in titer has occurred;* while the findings of two laboratories may not be in agreement with respect to the titer, they will generally be in agreement with respect to the magnitude of the titer increase encountered.

Neutralization Tests

When immune serum is added to its homologous virus, the infectivity of the virus is "neutralized," i.e., the mixture is noninfective for living susceptible cells. In the laboratory, the living susceptible cells for purposes of neutralization tests are supplied by embryonated hens' eggs, animals of various species, and by vertebrate, generally mammalian, cell cultures. The use of tissue cultures for neutralization tests is a relatively new development which in itself would warrant some discussion if space permitted.

There are two basic methods for performing neutralization tests. In the first method, the varying virus-constant serum method, the undiluted serum is tested against serial dilutions (decreasing amounts) of virus to determine the maximal amount of virus that can be neutralized. In the second method, the constant virus-varying serum method, serial dilutions of serum are tested against a standard amount of virus, for example 100 doses, in order to determine the minimal amount of antibody that will neutralize this standard test dose. Animals are inoculated by various routes depending upon the pathogenetic properties of the test virus employed and titration endpoints based on various types of reaction are used, such as mortality endpoints, infectivity endpoints, or lesion endpoints, etc. In the case of tissue cultures, two types of endpoints are used. These

are relatively new techniques. The first endpoint used in connection with neutralization tests performed in monolayer cell cultures is based on the detection of cytopathogenic effects (CPE) observed by microscopic examination of the cultures; the second type of endpoint is based on color changes in the indicator dye induced by metabolites accumulating in the culture tubes. Whatever the type of endpoint employed, allowance has to be made, as in the case of serologic methods, for an error inherent in all virologic methods which employ serial dilutions of reagents. Simply stated, the inherent error is such that variations of one dilution step may occur on replicate testing. Thus, in a system which employs serial tenfold dilutions, the results of replicate tests may vary one step upward or downward in the dilution series.

Neutralization tests are rather cumbersome and time consuming to perform, and since they employ animals or living tissues they are relatively expensive. Wherever possible, therefore, a simpler procedure such as complement fixation is given preference. However, the neutralization test does have certain very important applications. There are, for example, a number of viruses, such as some of the ECHO group, for which a satisfactory complement fixation test has not yet been obtained, and hence the neutralization test provides the only means for antibody assay. Another example is afforded by that group of agents known as the adenoviruses, a group comprised of a number of different viral serotypes. The agents in this group produce a common complement-fixing antigen so that while diagnosis of infection by any member of the group can be readily ascertained by means of the complement fixation test, neutralization tests are required if the specific serotype involved is to be identified.

3. VIRUS ISOLATION PROCEDURES

An obvious and important limitation to the serologic approach to diagnosis is that specific serologic tests can be performed only for those diseases whose causal virus has been isolated and can be cultivated in the laboratory. Consequently, virus isolation attempts are desirable in those obscure or vague illnesses in which the causation is unknown but is presumably viral. Such a presumption, unfortunately, is often reached late in the patient's illness, at a time when there is every reason to expect virus isolation attempts to be unsuccessful. Reference to Table 1 will show that virus is frequently demonstrable in appropriate test material taken at onset or during the early acute phase of the illness and only rarely during the recovery or convalescent phases. There are certain exceptions, notably poliomyelitis, but it is only reasonable to expect that as the patient's defense mechanisms are rallied and brought into full play during recovery, the offending virus will have been disposed of by the host and the opportunities for its recovery decrease with time.

As was mentioned earlier, attempts to recover the etiologic virus are frequently desirable in order to confirm diagnostic conclusions reached through microscopic methods. Another indication for virus recovery is the inability to make a differential diagnosis on serologic grounds between viruses closely related immunologically, e.g., variola and vaccinia. Finally, virus recovery frequently is necessary to determine definitively the etiology of outbreaks of disease of suspected viral causation. An example of this was the finding that epidemics of respiratory disease occurring during the late spring and summer of 1957 were due to an entirely new family of influenza viruses, the so-called Asian family. Another example lies in the relatively recent demonstration that aseptic meningitis constitutes a clinical syndrome caused by a diversity of viruses, among them the mumps virus and various immunologic serotypes in the Coxsackie and ECHO virus groups.

Various host systems are used for virus isolation purposes: embryonated hens' eggs; various animal species, especially the albino Swiss mouse; and a variety of vertebrate (primarily mammalian) cell types in culture. Successful recovery of a virus demands the use of a susceptible host and it is here that a clinical and epidemiologic history is helpful in guiding the virologist's choice of host systems to be used. Thus, in searching for influenza virus in a throat washing, the susceptible animal of choice would be the developing chick embryo and not strain HeLa carcinoma cells in culture. If, however, one had reason to suspect that an adenovirus might be implicated, the throat washing would be tested in cultures of HeLa cells, highly susceptible to infection with adenoviruses but insusceptible to the influenza viruses. Similarly, while the polioviruses can be isolated in cultures of either monkey kidney epithelial cells or strian HeLa carcinoma cells, enteroviruses of the ECHO family can be isolated in the former but not, so far as is known, in the latter cell culture system. The Coxsackie family of viruses affords another example. Some of these viruses can be readily isolated in monkey kidney epithelial cell cultures, but the majority cannot. All of these viruses, however, can be isolated in the albino Swiss mouse, but only very young suckling animals are susceptible, and the greatest success in isolations is attendant upon the use of infant mice less than 24 hours of age.

The use of infant mice and mammalian cell cultures has led to the discovery within recent years of whole new families of viruses comprised of a large multiplicity of serotypes. The identification and typing of these numerous serotypes has given rise to technical problems of considerable magnitude. The Coxsackie family consists of at least 24 serotypes, and presumably more, divided into two groups; the adenoviruses exist in a number of serotypes as indicated earlier; and the ECHO family of viruses consists of at least 24 serotypes. (ECHO is short for "enteric cytopathogenic human orphan," the "orphan" originally being applied because the etiologic relation, if any, of the agent to human disease was unknown.)

Since specific therapeutic measures are not available at the moment for treating infections caused by the ECHO, Coxsackie, or adeno- groups of viruses, knowledge as to the immunologic type of the infecting virus is not of prime importance clinically, but it is of the utmost importance in studying the spread of specific etiologic disease entities and from the standpoint of eventual prevention, control, and therapy.

The humble complement fixation test will serve adequately to reveal

that a patient is suffering from infection with an adenovirus, but if determination of the serotype involved is a requisite, then isolation and typing of the agent must be undertaken, or neutralization tests, which are highly type-specific, undertaken against the various serotypes known to be involved in human disease.

However, to use complement fixation tests for serologic diagnosis of a Coxsackie virus infection would entail performing a series of 24 or more complement fixation tests in an effort to determine whether this group of agents was involved. Aside from the fact that the complement fixation test is not highly reliable, there is also the very important economic aspect of antigen cost. It will be recalled that the Coxsackie viruses are cultivable only in suckling mice, and the cost of antigen prepared from the very small amounts of brain tissue obtainable from mice only several days of age is obviously quite high. To perform neutralization tests against a spectrum of 24 serotypes in order to determine to which one a rise in antibody titer has occurred is likewise a prohibitively expensive procedure. In the case of the Coxsackie viruses, therefore, it is simpler to attempt isolation of the virus from stool specimens using one-day-old mice as test animals, and then ascertain whether the patient has had a rise in antibody titer to the virus recovered from him. (There are some valid objections to this type of diagnostic approach, but space does not permit of discussion here.) A rough classification of the Coxsackie viruses into Group A or Group B can generally be made from the nature of the gross and microscopic lesions produced in the suckling test mouse.

Much of the same difficulty obtains with the ECHO viruses, since complement fixation tests either are not available or are unsatisfactory, and neutralization tests have to be employed. Since there are some 24 serotypes within the ECHO group, such tests would be economically prohibitive to almost any patient. As with the Coxsackie viruses, it would appear simpler to isolate the virus in a tissue culture system and determine whether the patient has had a significant rise in antibody titer to the virus isolated.

The picture I have painted thus far with respect to the typing of the numerous serotypes in the various virus families is indeed a highly pessimistic one, but it is mitigated by certain practical considerations. Some serotypes have not yet been associated with human illness, while others have been associated with certain syndromes. Some Coxsackie virus serotypes, for example, have been associated with herpangina, others with aseptic meningitis, certain ones with epidemic pleurodynia, some with myocarditis and pericarditis, etc. Knowledge of the patient's clinical history may thus give some clue as to the immunologic type of the infecting agent that might be involved and so serve to narrow the field with respect to the identification tests that will be required. This is not, of course, an infallible procedure, but it does have practical merit. Likewise, certain ECHO viruses have not yet been associated with human disease, whereas some serotypes have been implicated in aseptic meningitis, some in summar diarrheal disease of infants, and some in respiratory-enteric disease. Here too, this knowledge is of value in pointing to the probable types that might be involved and thereby indicating the identification tests that should be considered.

SUMMARY

This is a brief description and evaluation of three general approaches utilized in the laboratory diagnosis of viral diseases. That the viral diagnostic laboratory is confronted with a host of problems is quite evident. Some of these problems stem from lack of information concerning the patient's clinical history and physical findings; other problems are of a highly technical nature; and still others are matters of simple economics.

Limitations of methodology arise from our relative lack of knowledge of the properties and characteristics of some of the human pathogens, especially those in the several recently recognized virus families. As knowledge of these agents is gained, simple and effective diagnostic methods will undoubtedly be worked out.

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Los Angeles Health Centers Hold Open House

Los Angeles County and Los Angeles City Health departments have again joined to observe their 15th annual Community Health Week from March 15th through 22d.

Dr. George M. Uhl, Los Angeles city health officer, and Dr. Roy O. Gilbert, county health officer, report that the 23 health districts throughout the city and county will hold open house and feature special programs throughout the week. Participants will meet with national, state, and local health authorities. Voluntary organizations, public health agencies, and public and religious-sponsored schools are co-operating in developing health skits, demonstrations, and special exhibits.

Dr. Ruth Temple of the Los Angeles City Health Department is general chairman, and Dr. William F. Quinn, President of the Los Angeles County Medical Association is co-chairman.

Change of Health Officer

Arnold W. Brockmole, M.D., M.P.H., has been appointed health officer of Butte County, effective January 19, 1959, replacing Dr. Donald Hewitt, who was serving as health officer pro tem.

Since 1951 Dr. Brockmole has been assistant health officer, epidemiologist and director of communicable disease control for the Evansville-Vanderburgh County Health Department in Evansville, Indiana.

Dr. Brockmole recevied his M.P.H. degree at the Johns Hopkins School of Hygiene and Public Health.

Local Health Research Projects Encouraged by State Grants

Seventeen local health departments currently are engaged in 26 demonstration and research projects supported by state grants totaling $222,000 and covering the fields of maternal and child health, occupational health, aging and chronic disease.

The local projects encompass a broad range of public health interest, such as the demonstration of a home nursing program in a rural area, the study of gonadal exposure from medical radiation, a demonstration of patient restorative services in nursing homes, a rehabilitation nursing program for the disabled and elderly at Fairmont Hospital, evaluation of a hearing conservation program, and the distribution and characteristics of staphylococcal infections in a general hospital.

Purpose of the program is to focus attention on the changing needs in the field of occupational health, aging and chronic disease, and maternal and child health, to advance public health knowledge and practice and to encourage research in these fields through the efforts of local health departments.

In operation, a local health agency submits a project application to the State Department of Public Health. The applications are reviewed twice a year by an advisory committee composed of local health officers and representatives from schools of public health, schools of medicine, voluntary health agencies and private physicians.

The advisory group gives priority rating to approved applications and submits recommendations to the director for action. Applications are considered on the basis of clarity and worthiness of objectives, adequacy of proposed research methods, soundness of the plan for evaluation, and practicality of budget, staff and facilities.

Participating in the program are the county health departments of Yolo, Butte, Los Angeles, Shasta, San Bernardino, Contra Costa, Marin, San Joaquin, Santa Cruz, San Francisco, Alameda, San Mateo, Santa Clara and Modoc. Participating city health departments are Los Angeles, Long Beach and Berkeley.

Gov. Brown Appoints Two New Members To State Board of Public Health

Governor Edmund G. Brown has appointed to the State Board of Public Health Stephen I. Zetterberg, Claremont attorney, and Herbert A. Lints, M.D., Escondido physician. They replace Francis A. Walsh, Los Angeles pharmacist and Samuel J. McClendon, M.D., San Diego physician, whose terms have expired.

Mr. Zetterberg is President of the Board of Trustees of Casa Colina Rehabilitation Center for Crippled Children at Chino. He is also a member of the Board of the Claremont Medical Research Foundation.

After a year on a graduate fellowship at the National Institute of Public Affairs in Washington, D. C., Zetterberg took his LLB. degree at Yale in 1942.

Following brief service as a mediation assistant on the War Labor Board in Washington, Zetterberg was in the Coast Guard from 1942 to 1945. He was then legislative assistant to Illinois Senator Scott Lucas for one year before entering private law practice in Pomona in 1947.

Dr. Lints is president-elect of the staff of the Palomar Memorial Hospital in Escondido and is currently head of the hospital's committee on infection control. In 1958 he was chairman of the Heart Association's fund raising drive in Escondido.

Dr. Lints received his medical degree in 1945 from the University of Michigan Medical School and served his internship at the U. S. Naval Hospital in Astoria, Oregon, from 1945 to 1946. He was resident physician with the University of Chicago Clinic from 1948 to 1951. At the present time he is in private practice in Escondido and limits his practice to internal medicine.

County Health Departments Serve Three New Cities

Three newly incorporated cities in California are now being served by their county health departments. They are the Los Angeles County cities of South El Monte, incorporated in July of 1958, and Walnut, incorporated in January of this year, and Alameda County's Union City, also incorporated in January.

Medical Students Offered Summer Work in Public Health

The California State Department of Public Health is offering medical students opportunities for professional development through a paid summer work experience in public health and preventive medicine.

The students, selected from accredited medical schools, will temporarily increase the department's staff and make it possible for ongoing public health programs to be accelerated while the students are gaining a broader perspective of the practice of medicine.

These students can make valuable contributions to the Department's current studies of air pollution health effects, alcoholism, social factors in health, child health supervision, diagnostic and research laboratory procedures, surveillance of neurotropic virus diseases, occupational health, nutritional status of the aging, epidemiology of childhood accidents, epidemiology of heart disease, cancer, prevention of blindness, and reproductive loss (especially prematurity and congenital malformations).

During the period of training the students will serve as Medical Student Assistants and will receive $310 to $395 per month, depending on educational background.

Drs. Lennette and Johnson Contribute to Book

Two members of the California State Health Department's Viral and Rickettsial Disease Laboratory have contributed chapters to the 1959 edition of "Viral and Rickettsial Diseases of Man." This is the third edition of this book, edited by Rivers and Horsfall and published by J. B. Lippincott Company of Philadelphia.

Dr. Edwin H. Lennette, chief of the laboratory, has contributed chapters on serologic reactions in viral and rickettsial infections and on Q fever. Dr. Harald Johnson has written a chapter on rabies.

Dr. Johnson, a member of the Rockefeller Foundation staff, has been on assignment to the department's viral and rickettsial disease laboratory for the last four years.

Board Adopts Ground Rules For Radioactive Tracer Use

The State Board of Public Health this month adopted a six-point policy covering the uses of radioactive materials in environmental tracer studies.

The policy was drafted following a report by the University of California that it is negotiating a research contract with the Los Angeles County Flood Control District to study the movement of recharged ground water in the San Gabriel Valley basin and coastal plain of Los Angeles County with the radioactive isotope tritium.

For a number of years the flood control district has spread flood water on the gravel beds at the mouths of the mountain canyons in the valley to recharge the underground basins.

The university proposes to trace the routes taken by the recharged water, the subbasins benefited by the recharge, and the rate of travel of the water to the Long Beach area by adding tritium to the flood water. The water that is spread will have a concentration of tritium only 1 percent of the maximum permissible concentration in drinking water.

Recognizing the importance of using radioactive materials for such research projects, the board's policy establishes criteria for the safe use of such materials. While not regulatory in nature, the policy sets forth guidelines to agencies planning such studies.

The following criteria were established:

1. There is shown to be a substantial public interest in the information intended to be obtained in the study.

2. The study will be performed by persons or agencies having competence to use such radioactive material with due regard to its effect on public health.

3. The study is planned to impose the least possible exposure to ionizing radiation on the population consistent with achieving the study's objective.

4. There is no likelihood that the exposure of any person will exceed the recommended permissible limits of radioactivity in air, water, food, or space as a result of the study.

5. Persons and agencies having a principal interest in the media being investigated in the tracer study have been adequately advised.

6. The study is carried out under license by the Atomic Energy Commission and under observation by official public health agencies.

The policy has been forwarded to all involved agencies in the proposed Los Angeles County tracer study of ground water movement.

PUBLIC HEALTH POSITIONS

Alameda County

Director of Mental Health Program: Salary, $1,250 per month. This is a new program. The director will be expected to organize the program to provide informational, educational, and consultative services on mental health. Requires California medical license, plus a certification as psychiatrist by the American Board of Psychiatry, or eligibility therefor.

Health Educators: Salary range, $415 to $481, or $481 to $556, depending on experience. Requires M.P.H. degree from an accredited school of public health, or college graduation, plus two years of public health education experience.

Public Health Medical Officer: Salary range, $891 to $950. To work as an administrator of a county health department bureau. Requires California medical license, plus one year of graduate public health education, or one year of medical experience in public health. Examination by interview only.

For further information regarding any of these positions write to Alameda County Civil Service Commission, 12th and Jackson Streets, Oakland 7, California, or phone HI gate 4-0844, Extension 255.

Contra Costa County

Health Educator: Salary range, $481 to $584. Requires master's degree in public health education. Job locations affords easy commute to Berkeley, San Francisco, etc. Combined retirement and social security benefits, medical plan, sick leave, and three weeks annual vacation. Apply to Contra Costa County Civil Service Commission, Box 710, Martinez, California, or phone AC-ademy 8-3000, Extension 415.

Mendocino County

Sanitarian: Salary range, $376 to $458. General sanitation program in rural area. Three weeks vacation and 15 days sick leave per year. Group insurance and retirement plan. $135 per month car and expense allowance. Requires California registration. Apply to David C. Long, R.S., Director of Sanitation, Mendocino County Health Department, P. O. Box 205, Ukiah, California.

Santa Cruz County

Director of Health Education: Salary range, $455 to $553. Requires a master's degree in health education, or college graduation and two years of health education experience. Applications accepted through April 3d. Apply to Personnel Director, County of Santa Cruz, 105 Soquel Avenue, Santa Cruz, California.

Director of Public Health Nursing: Salary range, $478 to $581. To organize and direct the county public health nursing program and to integrate nursing activities with the overall health department program. Requires California registration and a combination of training and experience equivalent to completion of a master's degree in public health, plus three years of public health nursing experience, one of which was under direct qualified nursing supervision, and one of which was in a supervisory or administrative capacity. Written test and interview required. Apply by April 10th to County of Santa Cruz Personnel Department, 105 Soquel Avenue, Santa Cruz, California.

San Diego County

Occupational Therapists and Physical Therapists: Salary range, $397 to $483. To administer occupational therapy or physical treatments to children under the care of the Crippled Children's Services of San Diego County. Requires graduation from a recognized school of occupational or physical therapy (depending on the position applied for) and one year of supervised experience. Preference given to those experienced in treating cerebral palsy. Occupational therapists must have membership in the National Registry of Qualified Occupational Therapists, and physical therapists must have certification of California registration before permanent appointment.

For further information and application for either of these positions write to County Personnel, Room 403, Civic Center, San Diego 1, California.

San Mateo County

Director, Public Health Nursing Service: Salary range, $681 to $825 (includes $100 from VNA). To direct the public health nursing service under a combined department of health and welfare, and to serve as executive director of the Visiting Nurse Association. Requires California registration, or qualification therefor; any combination of training and experience equivalent to completion of a master's degree in the field of public health, and five years of recent generalized public health nursing experience, two years of which must have been in a supervisory or administrative capacity. For further information and application form contact San Mateo County, Civil Service Commission, Courthouse, Redwood City, California. Telephone: EM erson 9-1441, Ext. 351.

WCLHO Organized

Wyoming held its first conference for local health officers early this year.

As reported in the February 15th issue of *Good Health for Wyoming*, "Probably the most important single development of the three-day meeting was the organization of the health officers into a group known as the Wyoming Conference for Local Health Officers."

State Health Department Chemists Receive Maximum Merit Awards

At the recent Berkeley meeting of the California State Board of Health, Dr. Charles E. Smith, President of the Board of Health and Dean of the University of California School of Public Health, presented maximum merit awards to two members of the State Health Department's Air and Industrial Hygiene Laboratory.

State merit award certificates and checks for $150 each were presented to Harmon Wong-Woo and Chester L. Gordon, assistant public health chemists, for developing methods of calibration and standardization for automatic recording equipment used in air pollution districts throughout the State in the surveillance of air pollution.

Laboratory Advisory Committee Responsibilities Broadened

The Advisory Committee to the State Health Department's Division of Laboratories was recently reorganized and its responsibilities broadened to include matters relating to policy and administration of clinical laboratory laws and regulations.

The committee, formed in 1941 to assist with examinations of applicants for clinical laboratory technologist and technician licenses, will continue this function and assist, as well, in the formulation of necessary regulations related to clinical laboratories.

The advisory group, once split into Northern and Southern California committees, was reorganized as a single committee and its membership reduced to six.

It is expected that the reorganization will provide an efficient professional advisory service to the department and to the some 8,000 bioanalysts and technologists and the 1,100 licensed hospital and other clinical laboratories in the State.

Dr. Felix J. Underwood Dies in Mississippi

Dr. Felix J. Underwood, Mississippi's first full-time health officer, died suddenly at the State Health Department in Jackson, Mississippi, on January 9th.

Dr. Underwood was recognized internationally as one of his era's most capable public health officials. At the time of his death he had retired from the position of state health officer which he had held for the past 34 years and was acting as state chairman for the March of Dimes.

In 1958 Dr. Underwood was named Mississippi's "Man of the Year." The newly constructed State Health Center in Jackson, Mississippi, has been named in his honor.

U. S.-Mexico Border Conference On Health to Meet

The sister cities of Brownsville, Texas, and Matamoros, Tamaulipas, Mexico, will be the setting for the seventeenth annual meeting of the United States-Mexico Border Public Health Conference to be held March 30th to April 3d.

Six sections will hold meetings. They are: Maternal and Child Health, Nursing, Sanitation, Veterinary Public Health, Venereal Disease, and Tuberculosis.

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